

CLAIMS

We claim:

1. A method of identification and quantification of amine in a sample comprising the steps of:
 - a) combining a known amount of an amide internal standard with said sample comprising said amine;
 - b) contacting said sample with an acid anhydride or an acid chloride to convert said amine in said sample into an amide of identical structure as that of said amide internal standard except for the stable isotope atoms;
 - c) extracting said sample to isolate said amide and said amide internal standard; and
 - d) analyzing said amide and said amide internal standard by mass spectrometry.
2. The method of claim 1 wherein the concentration of said amine in said sample is determined and quantified by isotope dilution mass spectrometry using isotope labeled internal standard.
3. The method of claim 1 wherein said amine is a primary amine or a secondary amine having the following formula R_1NH_2 and R_1R_2NH wherein R_1 and R_2 are alkyl, aryl, and heteroatom containing cyclic or non-cyclic groups.
4. The method of claim 1 wherein said amide internal standard is a stable isotope labeled internal standard.
5. The method of claim 1 wherein said amide internal standard is synthesized by reacting an authentic sample of said amine with a stable isotope labeled reagent to form said amide internal standard having the following formula R_1NHCOR_3 or $R_1R_2NCOR_3$, wherein R_3 is a stable isotope labeled alkyl or aryl group.

6. The method of claim 1 wherein the extraction step c) can be any appropriate separating methods such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction.
7. The method of claim 1 wherein said acid anhydride is selected from a group consisting of acetic acid anhydride, propionic acid anhydride, and benzoic acid anhydride.
8. The method of claim 1 wherein said acid chloride is selected from a group consisting of acetyl chloride, propionyl chloride, and benzoyl chloride.
9. The method of claim 1 wherein said sample contains either a singularity or a plurality of primary amines and/or secondary amines.
10. The method of claim 1 wherein there is no conversion of said stable isotope labeled amide internal standard to its corresponding non-labeled amide compound during step b).
11. The method of claim 1 wherein the converting step b) is performed in an aqueous environment.
12. The method of claim 1 wherein the converting step b) is performed before the extraction step.
13. The method of claim 1 wherein the converting step b) is quantitative.
14. The method of claim 5 wherein said stable isotope labeled alkyl group and aryl group are selected from a group consisting of CD₃, CD₂CD₃ and C₆D₅ respectively.
15. A method of identification and quantification of amine in a sample comprising the steps of:
 - a) combining a known amount of a carbamate internal standard with said sample comprising said amine ;
 - b) contacting said sample with a chloroformate to convert said amine in said sample into a carbamate of identical structure as that of said carbamate internal standard except for the stable isotope atoms;

c) extracting said sample to isolate said carbamate and said carbamate internal standard; and
d) analyzing said carbamate and said carbamate internal standard by mass spectrometry.

16. The method of claim 15 wherein the concentration of said amine in said sample is determined and quantified by isotope dilution mass spectrometry using isotope labeled internal standard.

17. The method of claim 15 wherein said amine is a primary amine or a secondary amine having the following formula R_1NH_2 and R_1R_2NH wherein R_1 and R_2 are alkyl, aryl, and heteroatom containing cyclic or non-cyclic groups.

18. The method of claim 15 wherein said carbamate internal standard is a stable isotope labeled internal standard.

19. The method of claim 15 wherein said carbamate internal standard is synthesized by reacting an authentic sample of said amine with a stable isotope labeled reagent to form said carbamate internal standard having the following formula $R_1NHCOOR_3$ or $R_1R_2NCOOR_3$, where R_3 is a stable isotope labeled alkyl or aryl group.

20. The method of claim 15 wherein the extraction step c) can be any appropriate separating methods such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction.

21. The method of claim 15 wherein said chloroformate is selected from a group consisting of methyl chloroformate, ethyl chloroformate and phenyl chloroformate.

22. The method of claim 15 wherein said sample contains either a singularity or a plurality of primary amines and/or secondary amines.

23. The method of claim 15 wherein there is no conversion of said stable isotope labeled carbamate internal standard to its corresponding non-labeled carbamate compound during the converting step b).

24. The method of claim 15 wherein the converting step b) is performed in an aqueous environment.

25. The method of claim 15 wherein the converting step b) is performed before the extraction step.

26. The method of claim 15 wherein the converting step b) is quantitative.

27. The method of claim 19 wherein said stable isotope labeled alkyl group and aryl group are selected from a group consisting of CD₃, CD₂CD₃ and C₆D₅ respectively.

28. A method of identification and quantification of amines in a sample comprising the steps of:

- a) combining a known amount of an urea internal standard with said sample comprising said amine;
- b) contacting said biological sample with an isocyanate to convert said amine in said sample into an urea of identical structure as that of said urea internal standard except for the stable isotope atoms;
- c) extracting said sample to isolate said urea and said urea internal standard; and
- d) analyzing said urea and said urea internal standard by mass spectrometry.

29. The method of claim 28 wherein the concentration of said amine in said sample is determined and quantified by isotope dilution mass spectrometry using isotope labeled internal standard.

30. The method of claim 28 wherein said amine is a primary amine or a secondary amine having the following formula R_1NH_2 and R_1R_2NH wherein R_1 and R_2 are alkyl, aryl, and heteroatom containing cyclic or non-cyclic groups.

31. The method of claim 28 wherein said urea internal standard is a stable isotope labeled internal standard.

32. The method of claim 28 wherein said urea internal standard is synthesized by reacting an authentic sample of said amine with a stable isotope labeled reagent to form said urea internal standard having the following formula $R_1NHCONR_3$ or $R_1R_2NCONR_3$, where R_3 is a stable isotope labeled alkyl or aryl group.

33. The method of claim 28 wherein the extraction step c) can be any appropriate separating methods such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction.

34. The method of claim 28 wherein said isocyanate is selected from a group consisting of methyl isocyanate, ethyl isocyanate and phenyl isocyanate.

35. The method of claim 28 wherein said sample contains either a singularity or a plurality of primary amines and/or secondary amines.

36. The method of claim 28 wherein there is no conversion of said stable isotope labeled urea internal standard to its corresponding non-labeled urea compound during the converting step b).

37. The method of claim 28 wherein the converting step b) is performed in an aqueous environment.

38 . The method of claim 28 wherein the converting step b) is performed before the extraction step.

39. The method of claim 28 wherein the converting step b) is quantitative.

40. The method of claim 32 wherein said stable isotope labeled alkyl group and aryl group are selected from a group consisting of CD₃, CD₂CD₃ and C₆D₅ respectively.

41. A method of identification and quantification of amine in a sample comprising the steps of:

a) combining a known amount of an thiourea internal standard with said sample comprising said amines;

b) contacting said sample with a thioisocyanate to convert said amine in said sample into a thiourea of identical structure as that of said thiourea internal standard except for the stable isotope atoms;

c) extracting said sample to isolate said urea and said urea internal standard; and

d) analyzing said thiourea and said thiourea internal standard by mass spectrometry.

42. The method of claim 41 wherein the concentration of said amine in said sample is

determined and quantified by isotope dilution mass spectrometry using isotope labeled internal standard.

43. The method of claim 41 wherein said amine is a primary amine or a secondary amine having the following formula R₁NH₂ and R₁R₂NH wherein R₁ and R₂ are alkyl, aryl, and heteroatom containing cyclic or non-cyclic groups.

44. The method of claim 41 wherein said thiourea internal standard is a stable isotope labeled internal standard.

45. The method of claim 41 wherein said thiourea internal standard is synthesized by reacting an authentic sample of said amine with a stable isotope labeled reagent to form said thiourea internal standard having the following formula R₁NHCSNR₃ or R₁R₂NCSNR₃, where R₃ is a stable isotope labeled alkyl or aryl group.

46. The method of claim 41 wherein the extraction step c) can be any appropriate separating methods such as solid phase extraction, liquid-liquid extraction or solid supported liquid-liquid extraction.

47. The method of claim 41 wherein the thioisocyanate is selected from a group consisting of methyl thioisocyanate, ethyl thioisocyanate, and phenyl thioisocyanate.

48. The method of claim 41 wherein the sample contains either a singularity or a plurality of primary amines and/or secondary amines.

49. The method of claim 41 wherein there is no conversion of said stable isotope labeled thiourea internal standard to its corresponding non-labeled thiourea compound during the converting step b).

50. The method of claim 41 wherein the converting step b) is performed in an aqueous environment.

51. The method of claim 41 wherein the converting step b) is performed before the extraction step.

52. The method of claim 41 wherein the converting step b) is quantitative.

53. The method of claim 45 wherein said stable isotope labeled alkyl group and aryl group are selected from a group consisting of CD3, CD2CD3, and C6D5 respectively.